the lack of reference materials that reflect real-world polymer types and particle morphologies presents a challenge in predicting MNP toxicology accurately. Additionally, we developed lab-produced particles via cryomilling as well as cryomilled oceanic plastics to assess the feasibility of producing more environmentally relevant test materials for toxicological effects studies. This study aimed to characterize both highly referenced commercial MNPs and lab-produced MNPs to determine the best fit-for-purpose test materials for toxicology research. **Methods:** Six highly referenced polyethylene (PE) and polystyrene (PS) commercial microspheres, as well as cryomilled pristine and oceanic PE and PS particles were evaluated for their suitability as toxicology reference materials using multiple characterization methods. Particle size and morphology were determined using laser diffraction (LD), optical microscopy, and scanning electron microscopy (SEM). Chemical composition, molecular weight, crystallinity, and melting temperature were assessed through Raman spectroscopy and Pyrolysis gas chromatography mass spectrometry (Pyro-GC/MS), size exclusion chromatography gel permeation chromatography (SEC-GPC), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA), respectively. Surface charge was measured via dynamic light scattering (DLS) and elemental analysis was performed via SEM with energy dispersive X-ray spectroscopy (EDX), inductively coupled plasma mass spectrometry (ICP-MS) and X-ray photoelectron spectroscopy (XPS). **Results:** LD, SEM, and spectrometry results of commercial microspheres agreed well for size distribution, morphology, and polymer type, respectively, with manufacturers specifications, whereas the cryomilled particles presented more fragment like morphologies. Recent studies have shown that microsphere particles are taken up by cells more easily than other particle morphologies (i.e., fibers or fragments, etc.) and can accumulate over time. Recent studies characterizing environmental MNPs demonstrate a prevalence of fibers and fragments over spheres; therefore, spherical morphology of commercial MNPs lack environmental relevance. The zeta potential of the surface of the commercial microspheres fell in the range of -13 and -50 mV, consistent with the findings of Weiland et al that demonstrated that increasing negative zeta potential of PS microspheres resulted in strong increases in adhesion and internalization in murine macrophages. SEM-EDX and ICP-MS elemental analysis indicated the presence of aluminum in the ppm range, as well as other inorganic contaminants, potentially added during manufacturing of the commercial microspheres, a finding that could potentially influence toxicological outcomes. Thus, particle morphology, surface charge, potential contaminants, among other characteristics, should be considered when determining the best fit-for-purpose MNPs for toxicological effects studies. **Conclusions:** This study underscores the critical need for thorough characterization of MNP properties to enhance the relevance of toxicology studies. Our findings highlight that commercially available microspheres may not fully represent real-world exposures, as environmental microplastics often exhibit a broader range of morphologies and compositions than currently available commercial microspheres. Integrating multiple characterization methods, including those assessing particle morphology and surface properties, is essential for toxicological research aiming to understand the behavior of MNPs in biological systems. Developing more environmentally relevant MNPs, such as cryomilled ocean-sourced plastics, offers a promising approach to represent human exposures more accurately and could serve as a vital step toward advancing toxicology studies of fit-for-purpose MNPs in real-world contexts.

4123 Toxicological assessment of porous silica nanoparticles: cytotoxicity, genotoxicity and immunogenicity

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Background and Purpose: Porous silica nanoparticles (PSNs) have a huge potential as drug delivery vehicles due to their high surface area, accessible silanol groups, tuneable pore and particle size, and accessible surface functionalisation. However, to ensure their safe use, this study conducted a comprehensive assessment of PSN toxicity, exploring various toxicological endpoints such as cytotoxicity, genotoxicity, oxidative stress and immunogenicity on three cell lines representing intravenous routes of entry of PSNs. **Methods:** In this study, the physicochemical characterisation of all PSNs was performed using dynamic light scattering (DLS), zeta potential (ZP), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and powder X-ray diffraction (PXRD). Lymphoblastoid TK6, monocytic THP-1 and liver cancer HepG2 cells were exposed to five different functionalised PSNs, including polyethylenimine (PEI), amine, carboxyl, thiol or silanol for 24, 48 or 72 hours at concentrations between 0-200 µg/mL. The cytotoxicity (% cell viability) was measured using the colourimetric MTT assay. Genotoxicity of the PSNs was analysed using the micronucleus assay, where the % micronuclei (Mn) in binucleate cells (Bn) indicated genotoxicity. Intracellular reactive oxygen species (ROS) generation was assessed using the 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) assay. The immunogenicity of the PSNs was studied using SDS-PAGE electrophoresis and western blotting to identify the activation of the complement pathways. **Results:** The results showed that TK6 cells showed no significant toxicity across all PSNs tested. PSN-PEI was most cytotoxic on THP-1 cells, with lowest cell viabilities of 49% and 36% observed at 100 µg/mL and 200 µg/mL, respectively. No significant toxicity was observed with TK6 or HepG2 cells at the same concentration and time point. Interestingly, PSN-amine was the most cytotoxic on HepG2 cells compared to the other two cell lines. The lowest cell viability observed with PSN-amine at 100 µg/mL and 200 µg/mL on HepG2 cells was 61% and 57%, respectively, at 72 hours. Meanwhile, with TK6 and THP-1 cells at the same concentration and time point, cell viability was more than 85%. PSN-Thiol was the least cytotoxic PSN on THP-1 and HepG2 cells, with cell viability above 85% at all concentrations and time points. The micronucleus assay showed genotoxicity with PSN-PEI across all cell lines as the % Mn/Bn was higher than the control, which correlated with cytotoxicity results in THP-1. The presence of DNA damage in TK6 and HepG2 in the absence of cytotoxicity suggests that PSN-PEI could induce subtle alterations that could result in aberrant cellular processes. Similarly, PSN-amine showed genotoxicity with HepG2 cells, which was higher than that of THP-1 cells, while TK6 showed no DNA damage. Of the three lines tested, only THP-1 cells showed genotoxicity with PSN-carboxyl. Similar results were also obtained with the H2DCFDA assay. Oxidative stress was generated most by PSN-amine and PSN-PEI in HepG2 cells. In THP-1 cells, it was by PSN-amine, PSN-carboxyl and PSN-PEI. In TK6 cells, it was by PSN-PEI. Immunogenicity studies showed PSNs binding to the C3b complement protein, suggesting complement activation by all the five PSNs. **Conclusions:** This study provides crucial insights into PSN toxicity and the correlation between different endpoints assessed. These insights are instrumental in ensuring the use of safe and effective nanocarriers for drug delivery. They also enhance our understanding of nanotoxicology, a critical aspect of nanotechnology.

4124 Exposure and *in vitro* hazard characterization of microplastic dusts released from machining of boron nitride nanotube-enabled composites along its lifecycle

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Background and Purpose: Boron nitride nanotubes possess unique electrical insulation and radiation shielding abilities for use in light weight ceramics and flame-retardant insulation applications. Enabling plastic and epoxy resin composites with nanomaterials can affect technological performance and particle release characteristics during use scenarios, including nano- and microplastic release. The current study aimed to characterize airborne particle release during controlled machining of BNNT-enabled epoxy composites, evaluate the effect of weathering on airborne particle release, and conduct an initial hazard characterization of collected respirable particulate using macrophages. **Methods:** Three different BNNT-enabled epoxy composites (0%, 1%, and 4% BNNT by weight) were sanded (n=3-5 repeats for each composite) in a controlled laboratory apparatus using zirconium aluminum sandpaper. Direct reading instruments were used to quantify released particle number and size distributions while offline filter samples assessed particle concentration, morphology, elemental composition, and BNNT protrusions by electron microscopy. Next, composites were weathered under UV light and water for 2,016 hours, followed by sanding described above. A subset of weathered composites underwent immersion testing with increasing amounts of disruptive energy to evaluate release of BNNT from the epoxy matrix. Lastly, respirable dust suspensions in cell culture were characterized to determine particle morphology and hydrodynamic size. Differentiated human macrophages were exposed to respirable BNNT composite particulate (0 - 20 µg/cm^2) in submerged culture for 24 hours and assayed for cytotoxicity, mechanism of cell death, reactive oxygen species, and mitochondrial membrane polarization. **Results:** 4% BNNT epoxy released significantly more particulate (41,000 particles/cm^3) than 0% and 1% composites (30,000 particles/cm^3). Respirable sized particles on collected filters were agglomerated averaging 3 microns in size and were 68% and 89% carbon-based on a number concentration and mass basis, respectively. BNNT protrusions were found on particle surfaces albeit in low number (0.2 - 2% with at least one BNNT protrusion). Weathering increased particle release of 4% composite during sanding (61,000 particles/cm^3) compared to non-weathered composite. Free release of BNNT was observed in weathered 4% BNNT epoxy samples under immersion and light sonication conditions. Collected respirable BNNT epoxy sanding dust exposure (Z_{avg} 1.73 - 1.93 µm) caused dose-dependent increase in cytotoxicity (i.e. LDH) and a 10-15% decrease in live cell count due to mixed pyroptosis and apoptosis in human macrophages. A dose-dependent increase in intracellular reactive oxygen species and no change on mitochondrial membrane polarization were also observed. No clear difference between BNNT % loading and macrophage response was observed. **Conclusions:** Our current findings indicate that BNNT % loading and weathering affects particle number release during machining processes in part due to the number and strength of BNNT surface bonding with the epoxy matrix. Percent loading of BNNT had minimal effect on human macrophage response to machined respirable epoxy particulate.

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